P-042 **Copaiba oil microemulsion containing ketoconazole: Assessment of the rate of encapsulation**

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INTRODUCTION AND OBJECTIVE

Colloidal drug delivery systems are becoming more and more interesting because they enable controlled drug release and improved bioavailability (Podlogar 2004). Microemulsions (MEs), optically transparent systems with low viscosity, are thermodynamically stable dispersions of oil and water. They are stabilized by an interfacial film of a surfactant, usually in combination with a co-surfactant. In pharmaceutics, MEs has been used as carriers to deliver a number of drugs due to their thermodynamic stability, simple preparation and good appearance. (Podlogar et al., 2004)

Ketoconazole (KTZ) cis-1-acetyl-4-[4-[2-(2,4-dichloro phenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxa lan 4-yl] methoxy]phenyl] piperazine is an imidazole anti-fungal agent used in the treatment of superficial and systemic fungal infections. It acts by blocking the synthesis of ergosterol, an essential component of the fungal cell membrane. In the treatment of superficial and localized infection, topical application of antifungal seems to be an appropriate strategy to restrict the therapeutic effect to the affected area and to reduce the systemic incrimination (Lawrence 2000).

Encapsulation of KTZ in MEs may increase its half life, providing prolonged drug delivery and minimize the commonly occurring side effects (Patel 2010). Therefore, the aim of this work was to develop MEs Copaiba oil and assess the rate of incorporation of KTZ at these systems.

MATERIALS

The Copaiba oil was obtained from Flores & Ervas (Piracicaba, SP, Brazil), Tween 20 and KTZ was purchased from VETEC (Rio de Janeiro, RJ, Brazil). Ultrapure water was used throughout the experiments (Milipore® systems). All chemicals were of pharmaceutical grade and used as received without further purification.

METHODS

Production of MEs

The ME was produced by mixing its components (Table 1) under moderate stirring using vortex at 1500 rotation for 4 minutes. The production was based on a pseudo ternary diagram (Figure 1).

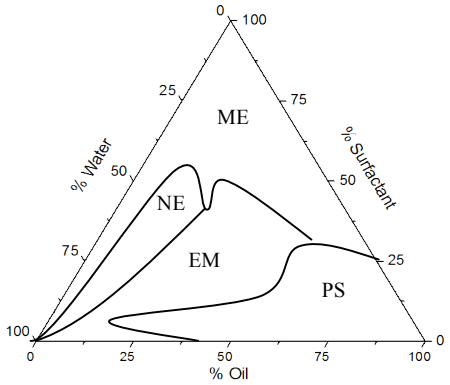

Figure 1 : Pseudo ternary phase diagram of Copaiba Oil systems. Where: ME, Microemulsion; EM, emulsion; NE, nanoemulsion; PS, phase separation.

Table 1 : ME-KTZ of Copaiba oil

Reagents	ME % (w/w)	ME-KTZ % (w/w)
KTZ	-	2.0
Copaiba Oil	20.5	20.5
Tween 20®	48.5	48.5
Water	29.0	29.0

Characterization of Systems

The color, isotropy and homogeneity of the MEs, and the presence of precipitates or phase separation were scored after visual and cross-polarized light microscopy evaluation. The observed pH values of the samples at 5% was measured by a PG-2000 pHmeter (GEHAKA, Morumbi, SP, Brazil), at 25 ± 2°C.

Analitical calibration Curve of KZT

The analytical calibration curve of KTZ was performed using a variation of concentration between 0.00614 and 0.01980 mg/mL in methanol using spectrophotometer UV/VIS at 246nm (Purchase Biochrom: Model Libra S32) using the stationary cuvette method (100mm) (Silva 2009).

Determination of encapsulation efficiency of KZT in ME

The ME-KTZ was centrifuged at 11.400g for 30 minutes at 25°C (Fanen, São Paulo, SP, Brazil). The supernatant were diluted in methanol and analyzed in spectrophotometer at 246nm. The encapsulation value was obtained according to equation: *%Encap = (KTZs x 100)/KTZt*.

Where KTZs is the concentration value of the KTZ in supernatant and KTZt is the KTZ concentration into the bulk of ME-KTZ.

RESULTS AND DISCUSSION

From the visual analysis no precipitates or color changes, as well as creaming and viscosity was observed.

From the pseudo ternary study, the formation of ME systems (the shaded area) was observed at room temperature. No liquid crystalline structure was observed using a cross polarizer. Phase behavior evaluation of this system demonstrated to be a suitable approach to determine the compounds concentration in which the ME system is formed.

The pH value of the ME-KTZ and ME were 6.8 ± 0.2, which is an optimum pH value for formulations for healthy skin. These constant values may indicate low rate of system degradation caused mainly by lipid peroxidation of components of oil and surfactant.

The calibration curve was performed in triplicate and produced both low Mean Standard Deviation and great precision, $R^2 = 0.999$. The regression equation was found to be Y = 0,035x - 0,045 (Figure 2).

The quantification of KTZ in ME systems is an important assay for the quality control of ME systems. In fact, there is a growing concern with the constant antimicrobial resistance, which could happen if the drug were at concentrations below the standard, or if toxicity was greater than usual. The encapsulation efficiency of the ME-KTZ system was 80 ± 4,82%. This value shows that w/o emulsion systems can be a viable alternative in the formulation of a topical preparation of KTZ in the skin.

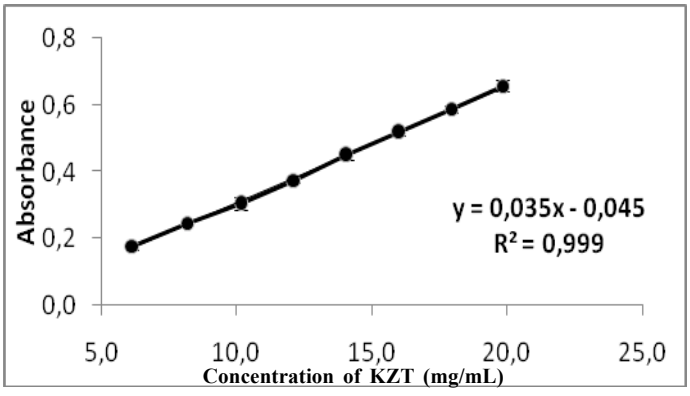

Figure 2 : Calibration curve for KTZ using the stationary cuvette method (Silva 2009).

CONCLUSION

To produce ME systems for topical delivery of KTZ, a suitable oil and surfactant has to be chosen. In this context the Copaiba oil shows to be a valuable ingredient for its good rate of incorporation, as for its intrinsic antifungal activity. The samples, which were examined by ocular inspection in a cross polarizer microscopy for homogeneity and birefringence, showed isotropy, which is a conclusive feature for ME systems.

The results indicated that both KTZ-ME and ME were optically isotropic colloidal dispersions. Both ME systems were transparent and presented as a homogenous single-phase liquid when observed their visual clarity against strong light. No traces of precipitated drug or other solid ingredient were found in all samples.

Previous stability studies show that this ME system is quite stable. Additionally, the KTZ encapsulation rate of 80% is perfectly acceptable for a therapeutic carrier. The future goal of our research is, however, increase this rate to 100%. Therefore, other ME areas of the pseudo ternary diagram will be evaluated. Also, the absence of color change into the ME-KTZ system may be indicative of the low degradation of the drug, which can be justified by the antioxidant properties of the oil phase of the system.

As future perspectives two other points will be evaluated: (i) to perform confirmatory test of antifungal activity and check the intrinsic activity of the ME due to the oil phase; (ii) to perform the long term shelf life stability.

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